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Evaluation of Microcirculation in the Tumor-Bearing Liver of Rabbits by Laser-Doppler Flowmetry

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Summary

Hemodynamic changes in normal liver tissue and in intrahepatic tumors (Vx2 carcinoma) after occlusion of the hepatic arterial branch or the portal branch (ex. 1), and with intrahepatic arterial infusion of vasoactive agents (ex. 2) were studied in rabbits by a laser-Doppler flowmeter. Ex. 1: After occlusion of the hepatic arterial branch to the main lobe, laser-Doppler flow (LDF) in main lobe normal tissue decreased by 11±11% in the control group, 37±37% in group Ia (tumors were 10–20 mm in diameter) and 49±37% in group Ib (tumors were 25–50 mm), so it seemed that the proportion of portal blood flow in the normal tissue microcirculation decreased with tumor growth. The tumor LDF decreased by 88±13%. After occlusion of the portal branch, the normal tissue LDF in the main lobe decreased and then recovered slightly (most evident in the control group and least in group Ib). This recovery was probably due to the hepatic arterial buffer response. The tumor LDF decreased by 36±10% in group Ia and 11±17% in group Ib. There was no difference between group I (tumors were implanted directly) and group II (tumors were implanted via portal vein). Ex. 2: Adenosine and prostaglandin E_1 increased blood flow in the normal tissue and decreased the tumor blood flow, while angiotensin II had the opposite effect. Vasoactive agents can be used to selectively increase or decrease tumor blood flow and are available as adjuvants for the treatment of liver tumors. Adenosine may enhance the selective tumor heating in local hyperthermia.

Introduction

In the therapeutic management of malignant tumors, their microcirculation and the vasculature should be taken into consideration, as well as the nature of the tumor cells per se. In the liver, which has a dual blood supply, ischemic therapy, including hepatic arterial branch ligation (8), portal branch ligation (11) and transcatheter hepatic arterial embolization (TAE) (21) has been used to

Key words: Laser Doppler flowmetry, Vx2 Carcinoma, Hepatic arterial branch ligation, Portal branch ligation, Adenosine.
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treat tumors. It is recognized that hepatic tumors are predominantly nourished by hepatic arterial blood. However, the margins of these tumors might be nourished by portal blood (11) which could in part explain the limited utility of TAE. *Ackerman* reported on the role of the portal circulation in hepatic tumor vascularity (1). On the other hand, it has been reported that tumor vasculature is quite different from that of normal tissue both anatomically and functionally, and responds differently to vasoactive agents and to physical stimuli, i.e. temperature (2, 6). These characteristics of the hepatic circulation and of tumor vasculature should be considered in the treatment of liver tumors.

The microcirculation of the liver and of liver tumors has been studied in both animals and humans with various techniques. Laser-doppler flowmetry allows continuous, noninvasive and instantaneous recording of spontaneous changes in the microcirculation of many tissues (5). We used this technique in rabbits to study the hemodynamics of normal liver tissue and liver tumors. We first measured changes in blood flow during temporary occlusion of the hepatic arterial branch and portal branch. Using these data, we first evaluated the ratio of hepatic arterial flow to portal flow and the relationship between them. We also studied the microcirculation of normal liver tissue and liver tumors, and the changes in microcirculation with tumor progression. Second, we studied the responses to vasoactive substances (adenosine, prostaglandin E_1 and angiotensin II). Angiotensin II has been used to selectively increase tumor blood flow, i.e. "hypertensive chemotherapy" (20). Adenosine and prostaglandin E_1 are potent vasodilators, and the microcirculation of tumors would be expected to responds quite differently to these substances, compared to normal liver tissue microcirculation. Since implantable arterial access systems (16) have simplified intrahepatic arterial infusion, modification of tumor microcirculation with vasoactive agents will probably be more frequently used to enhance the effects of cancer treatment.

Materials and Methods

A. Experiment 1

Twenty-three male adult rabbits weighing 1.7–3.4 kg (mean weight 2.4 kg) were used. Vx2 carcinoma cell suspension in calcium-free phosphate buffer (cell density of about 10^6/ml) was prepared from the Vx2 tumor maintained serially in the thigh muscle of rabbits. Under pentobarbital sodium anesthesia (25 mg/kg given intravenously), the rabbits underwent a small laparotomy by upper midline incision. In 13 rabbits (group I), Vx2 carcinoma cell suspension (0.2 ml) was injected directly into the hepatic parenchyma near the diaphragmatic surface in the left medial lobe (or the left lateral lobe in some rabbits). Cell suspensions were injected into the jejunal vein in 7 rabbits (group II). On the 10–21st day after implantation, the rabbits were anesthetized again and underwent laparotomy for hemodynamic studies with a laser-Doppler flowmeter (LD5000 Capillary Perfusion Monitor, Med-Pacific, Seattle). Group I was subdivided into group Ia, in which hemodynamics were studied on the 10–14th day after implantation ($n=5$) and group Ib in which studies were performed on the 15–21st day after implantation ($n=8$). In group II, hemodynamic studies were performed on the 10–18th day. The other three rabbits (control group) underwent laparotomy for hemodynamic study without injection of Vx2 carcinoma.

After 24 hours of a water-only fast the rabbits were anesthetized with pentobarbital sodium (25 mg/kg given intravenously, 2–5 mg/kg was added if necessary during the experiment), intubated with a tracheal tube by tracheostomy, and ventilated with a ventilator (Model SN-480-6, Shinano Co., Japan). The CO_2 concentration in end-expiratory gas was kept at 4.0–5.5% (15–25

ml × 15–25/min). A heating mat and lamp were used to keep the intraabdominal temperature between 37 and 38°C. The right femoral artery was cannulated with a 21 G catheter, and the mean arterial pressure was continuously monitored with a pressure transducer (Gould P23 ID) and recorded (PMP3004, Nippon Koden). The left femoral vein was cannulated with a 23 G catheter and physiological saline (10–15 ml/kg/h) was infused. To paralyze the rabbits, pancuronium bromide (0.05–0.1 mg/kg) was injected through this cannula every 30 minutes.

The abdomen was opened by a bilateral subcostal incision. The xiphoid process was excised and the falciform ligament was cut to expose a large part of the liver surface. The open abdomen was covered with a transparent vinyl sheet to prevent the abdominal viscera from drying.

In some rabbits in group I, before performing any other procedures, the microcirculatory blood flow of normal liver tissue was measured at several points in each lobe and in the tumor for about 5 minutes at each point with the LD5000. The DC (direct current of backscattered light) level of the LD5000 was adjusted, and a zero calibration was performed before monitoring the blood flow. When monitoring the blood flow, the needle-type probe of the LD5000 with a gum-sheet attachment device of our own design was softly placed on the surface of the liver (usually on a diaphragmatic surface), and the fiber cable was supported to prevent the probe from moving or compressing the tissue (Fig. 1). The LD5000 continuously monitors microcirculatory blood flow, and the output is expressed in mV (laser-Doppler flow parameter (LDF)). The LDF is proportional to the flux of red blood cells in a unite volume of about 1 mm^3. During the experiment, LDF and DC were recorded with a time constant of 1 sec (Watanabe servocorder SR652) at a paper speed of 15 mm/min.

The hepatic arterial branch and the portal branch to the main lobe were identified and dissected

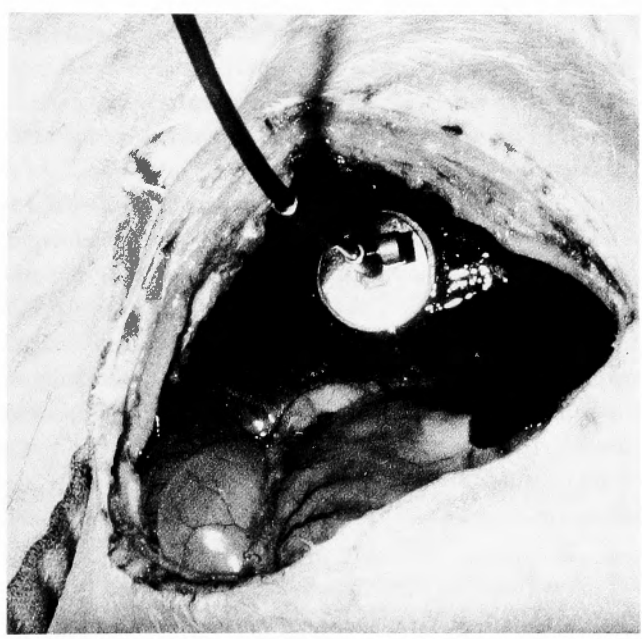

Fig. 1 Measurement of liver microcirculation in a rabbit with a laser-Doppler flowmeter (LD5000): the needle-type probe of the LD5000 with a gum-sheet attachment device is softly placed on the diaphragmatic surface of the liver.

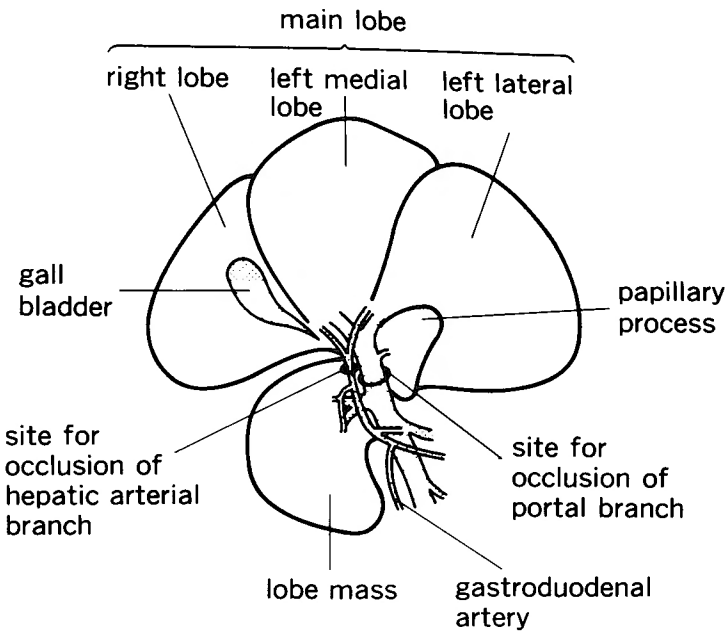

Fig. 2 Schematic representation of the rabbit liver and its blood supply, showing the sites for occlusion of the hepatic arterial branch and the portal branch.

from surrounding structures, and each vessel was encircled with a fine thread. (The rabbit liver is composed of a main lobe and a lobe mass. The main lobe is composed of a left lateral lobe, a left medial lobe, a right lobe and a papillary process.) (Fig. 2)

After at least 20 minutes, hemodynamic measurements were started. The sites of the measurements were several points on the exposed tumor, in the left medial or left lateral lobe of normal liver tissue, and at one point of normal liver tissue in the lobe mass. At each point, LDF was continuously measured while the hepatic arterial branch or portal branch to the main lobe was occluded for 3 minutes by pulling the thread encircling the vessel or by clamping the vessel with a microvascular hemoclip. At least 10 minutes after declamping a vessel, the other vessel was occluded for 3 minutes.

After the hemodynamic studies were completed, rabbits were killed by an overdose of pentobarbital sodium. In some rabbits LDF recordings were continued for 10–15 minutes after cardiac arrest to determine the true zero level, since it has been reported that LDF measured on tissues in which blood flow was completely occluded never reached the zero level. This is probably due to interference from some signal other than the flux of red blood cells. The size of the tumor was measured in every rabbit.

B. Experiment 2

Seven male adult rabbits weighing 2.0–2.4 kg (mean weight 2.2 kg) were used. Vx2 carcinoma cell suspension was injected directly into the main lobe, and on the 11–14th day after implantation, the rabbits underwent laparotomy for hemodynamic studies as in experiment 1. After laparotomy, the gastroduodenal artery was identified and cannulated with a 24 G catheter and the right gastric artery was ligated. Heparinized physiological saline was infused through this catheter to the hepatic

artery at 0.71 ml/hr except while vasoactive substances were being infused.

The LDF was measured on the tumor and then on the normal liver tissue in the main lobe. At each site, the effects of intrahepatic arterial infusion of adenosine (20 μg/kg/min and 100 μg/kg/min), prostaglandin E_1 (0.1 μg/kg/min and 0.5 μg/kg/min) and angiotensin II (0.2 μg/kg/min and 1.0 μg/kg/min) were recorded. These vasoactive substances were diluted with physiological saline and infused into the hepatic artery through the gastroduodenal artery catheter, at 0.15 ml/min for 10 minutes. Between each two successive infusions of vasoactive substances, there was a delay of at least 20 minutes to eliminate the effects of previously infused substances.

C. Statistical analysis

Continuous analog LDF and mean blood pressure data were digitized by averaging the data for every 5 seconds, to eliminate the ventilatory fluctuation. Basal LDF was calculated by averaging LDF values for 2 minutes just before occluding a vessel or before administration of vasoactive agents. %LDF was calculated with the basal LDF as 100% and the mean value of post-mortem LDF as 0% (see below). Mean %LDF flow curves (Fig. 4–8) were obtained by averaging %LDF data for each time point. The data were expressed as mean±S.D. Paired and unpaired Student's t-tests were used and the null hypothesis was rejected when $p<0.05$.

Results

A. Experiment 1

1. Sizes of tumors

The diameter of implanted liver tumors was 10–20 mm (15.8±3.3 mm) in group Ia, and 25–50 mm (39.6±9.3 mm) in group Ib. In group II, there were multiple nodules of Vx2 tumor in every lobe of the liver and their diameters were 1–8 mm.

2. LDF of normal liver tissue in dead rabbits

The LDF measured on the liver surface after the rabbits were killed was 27.1±14 mV, so we assumed that 27 mV was the true value for zero blood flow, and %LDF data were calculated as follows.

$$\%\text{LDF} = 100 \times (\text{LDF} - 27)/(\text{basal LDF} - 27)$$

3. Basal LDF of normal liver tissue and tumor

The LDFs measured on normal liver tissue and on liver tumors in group I rabbits before the vessels were prepared for occlusion are shown in Table 1. (These data are averages of each rabbit's averaged data for each lobe.) The LDF of normal liver tissue was highest in the lobe mass (153±54 mV) and lowest in the left lateral lobe (87±33 mV), and the difference was statistically significant between them. The LDF of the tumor was 158±68 mV

Table 1. LDF of normal liver tissue and tumor in group I measured before the vessels were prepared for occlusion.

normal liver tissue (mV)				tumor (mV)
lobe mass	right lobe	left medial	left lateral	left medial (or lateral)
153±54	121±51	108±33	87±33	158±68
		*		

(n=6, mean±S.D. * $p<0.05$)

LDF: laser-Doppler flow (blood flow parameter measured by laser-Doppler flowmeter).

Table 2. Basal LDF of normal liver tissue and tumor just before occluding the hepatic arterial branch or the portal branch.

		normal liver tissue (mV)		tumor (mV)
		lobe mass	main lobe	main lobe
control group	(n=3)	138±44	123±20	—
tumor bearing group (group I+group II)	(n=19)	153±57	124±31 *	204±114
group Ia	(n=5)	139±54	111±32	243±72
group Ib	(n=8)	150±56	128±32	171±112
group I (group Ia+group Ib)	(n=13)	146±53	121±32	201±101
group II	(n=6)	169±70	129±32	209±148

(mean±S.D. * $p<0.05$)

Basal LDF data just before occluding a vessel are shown in Table 2. In the control group, the LDF of the main lobe was 123±20 mV and that of the lobe mass was 138±44 mV In rabbits with tumors, the LDF of normal liver tissue in the main lobe was 124±31 mV, and this was significantly lower than that of the lobe mass (153±57 mV). The LDF of the tumor was 204±114 mV, and was more variable than that of normal liver tissue.

There was no significant difference between group I and group II. Comparison between group

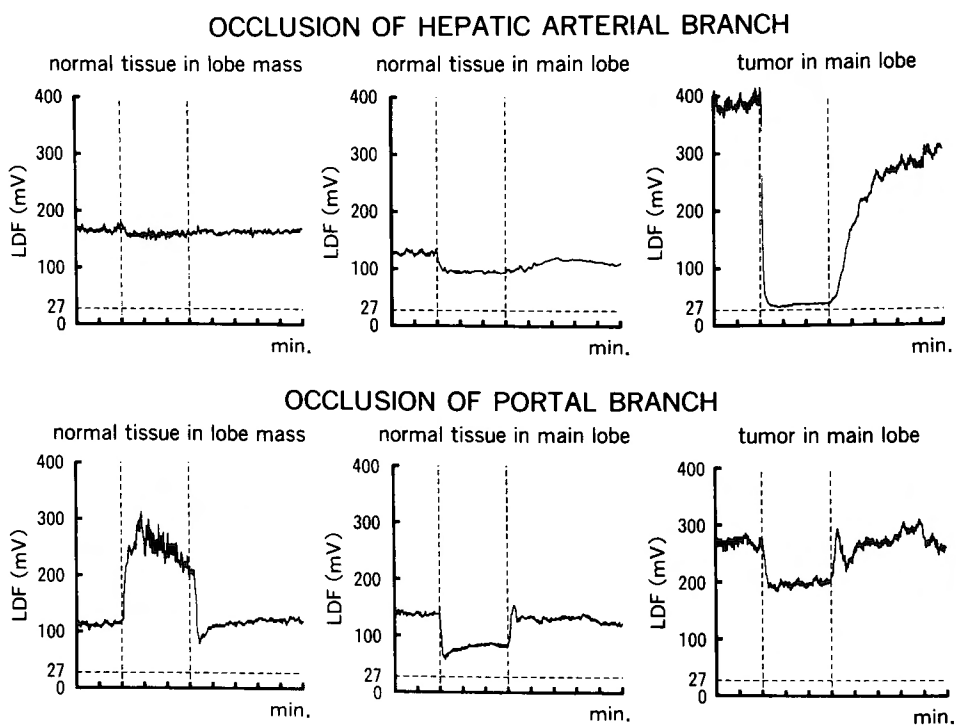

Fig. 3 Records of the laser-Doppler flow in normal liver tissue and in a tumor in group Ia (tumor size was 15×10 mm) with occlusion of the hepatic arterial branch or the portal branch for three minutes.

Ia and group Ib showed no significant difference, but there was a tendency for the tumor LDF to be higher in group Ia than in group Ib.

4. Changes in LDF after occlusion of the hepatic arterial branch and the portal branch

Results from a rabbit in group Ia (tumor size was 15 mm × 10 mm) are shown in Fig. 3. The LDF of the tumor in the main lobe promptly decreased almost to the zero level after occlusion of the hepatic arterial branch, and decreased to about 60% of basal blood flow after occlusion of the portal branch. The LDF of normal liver tissue in the main lobe decreased and reached its minimum at 15 sec after occlusion of the portal branch, and then slightly recovered to 45% of basal flow 100 sec after occlusion. Since this pattern of LDF change after occlusion of the portal branch was seen in many cases, we calculated the mean ratio of the change between 120 sec and 180 sec after occlusion as an average ratio of change in a plateau state (%ΔLDFavr), as well as a ratio at the maximum change (%ΔLDFmax), as follows:

%ΔLDFavr = average %LDF between 120 sec and 180 sec after occlusion − 100

%ΔLDFmax = %LDF at maximum change after occlusion − 100

4a. Changes in LDF after occlusion of the hepatic arterial branch (Fig. 4, Table 3)

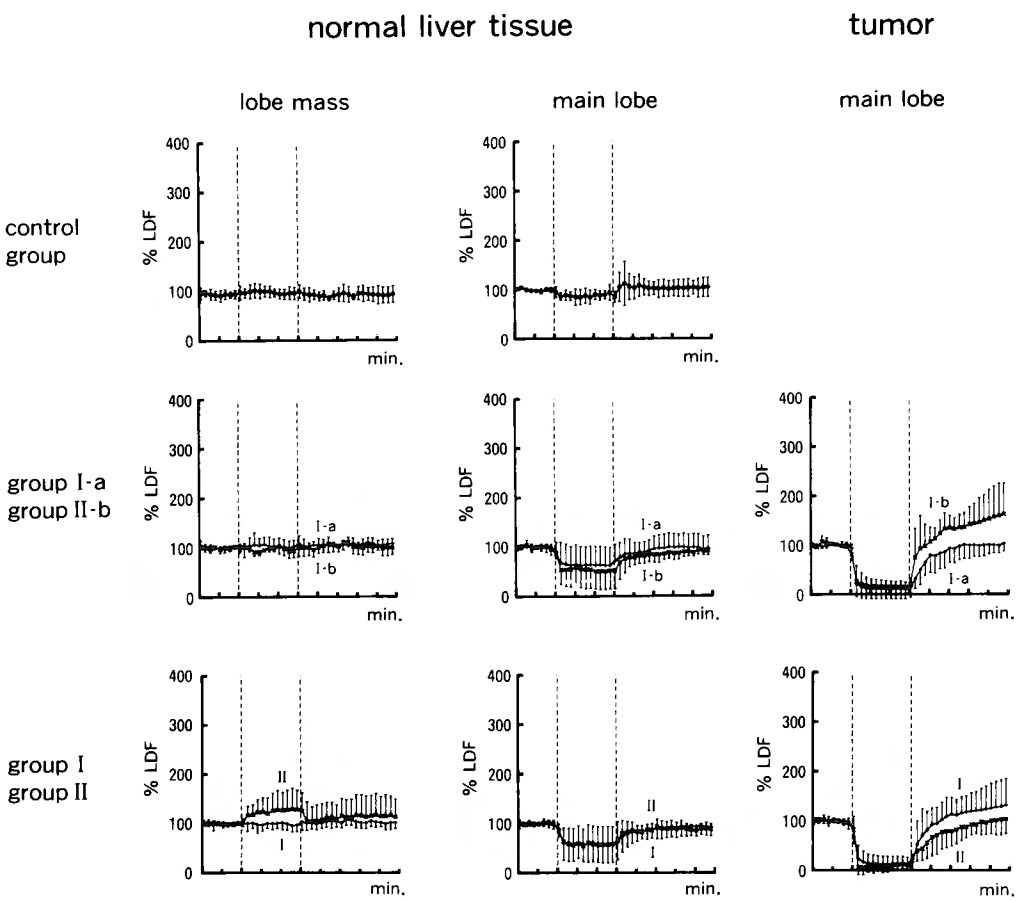

Fig. 4 Mean %LDF curves of normal liver tissue and a tumor, with occlusion of the hepatic arterial branch for three minutes.

Table 3. Changes in the LDF of normal liver tissue and tumor after occlusion of the hepatic arterial branch to the main lobe.

	normal liver tissue				tumor	
	lobe mass		main lobe		main lobe	
	%ΔLDFmax	%ΔLDFavr	%ΔLDFmax	%ΔLDFavr	%ΔLDFmax	%ΔLDFavr
control group	12±7	2±3	−20±14	−11±11	—	—
group with tumors	23±28	11±13	−49±33	−43±35	−92±15	−88±13
group Ia	19±14	1±16	−41±35	−37±37	−91±20	−89±20
group Ib	9±11	−3±13	−55±36	−49±37	−88±10	−86±12
group I	14±13	−1±13	−48±35	−43±36	−89±15	−87±15
group II	36±39	28±40	−50±33	−41±36	−96±16	−92±12

(mean±S.D.)

%ΔLDFmax=100×(LDF at maximum change after occlusion−basal LDF)/(basal LDF−27)
%ΔLDFavr=100×(average LDF between 120 sec and 180 sec after occlusion−basal LDF)/(basal LDF−27)

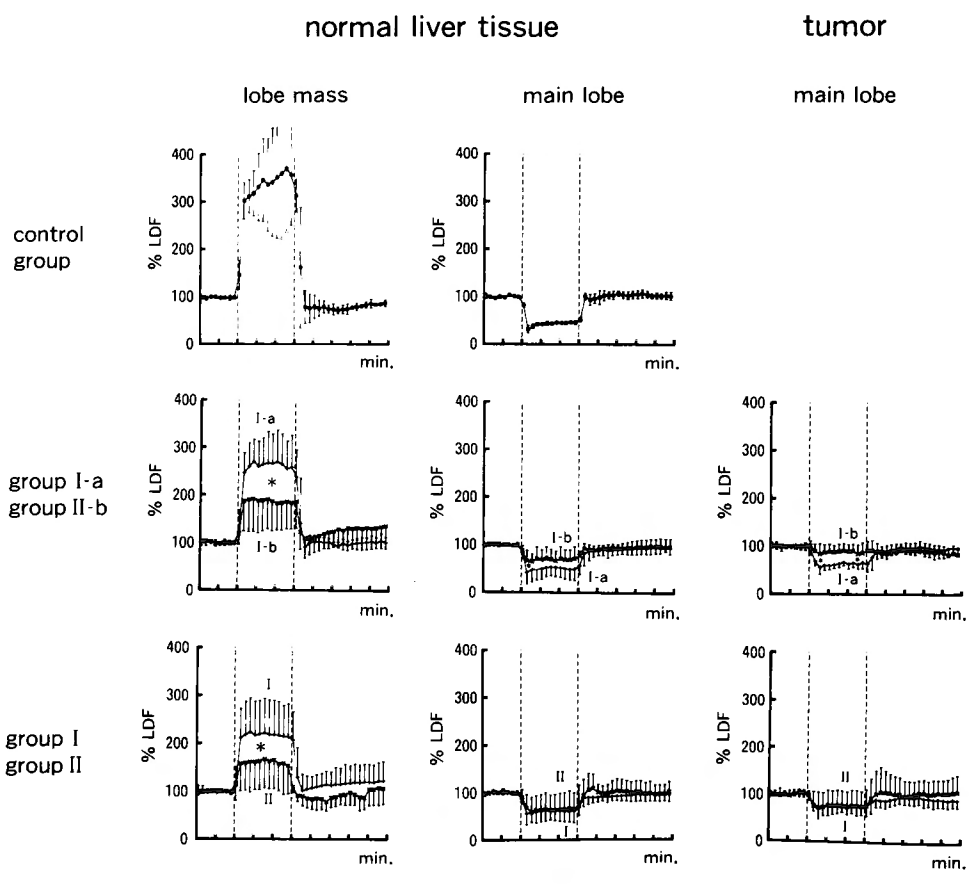

Fig. 5 Mean %LDF curves of normal liver tissue and a tumor, with occlusion of the portal branch for three minutes.

Table 4. Changes in the LDF of normal liver tissue and tumor after occlusion of the portal branch to the main lobe.

	normal liver tissue				tumor	
	lobe mass		main lobe		main lobe	
	%ΔLDFmax	%ΔLDFavr	%ΔLDFmax	%ΔLDFavr	%ΔLDFmax	%ΔLDFavr
control group	284±124 **	254±112 **	−70±7	−53±3 *	—	—
group with tumors	125±73 *	96±69 *	−53±23	−36±27	−35±23	−23±24
group Ia	199±54 *	162±63 *	−68±22 *	−49±22	−47±12 *	−36±10 *
group Ib	115±58	85±57	−42±17	−30±22	−23±18	−11±17
group I	149±69 *	116±69	−53±22	−38±23	−35±19	−23±18
group II	77±60	57±58	−52±26	−34±36	−35±30	−24±33

(mean±S.D. * $p<0.05$ ** $p<0.01$)

%ΔLDFmax = 100 × (LDF at maximum change after occlusion − basal LDF)/(basal LDF − 27)

%ΔLDFavr = 100 × (average LDF between 120 sec and 180 sec after occlusion − basal LDF)/(basal LDF − 27)

The LDF of normal liver tissue in the main lobe slightly decreased after arterial branch occlusion in the control group (%ΔLDFavr = −11+11%), and decreased by 37±37% in group Ia, 49±37% in group Ib and 41±36% in group II. The LDF of the lobe mass was not changed by occlusion of the hepatic arterial branch in the control group or in group I. In group II, the LDF of the lobe mass showed a slight increase, but it was not significant.

The LDF of the tumor in the main lobe promptly decreased to a very low flow level after occlusion of the hepatic arterial branch and stayed at that level without fluctuation until the occlusion was stopped. There was only one exception, a rabbit in group Ia in which LDF decreased by only 66%. In total, %ΔLDFmax was −92±15% and %ΔLDFavr was −88±13%.

4b. Changes in LDF after occlusion of the portal branch (Fig. 5, Table 4)

In the control group, the LDF in normal liver tissue in the main lobe reached its minimum at 15 sec (%ΔLDFmax = −70±7%) and then recovered slightly (%ΔLDFavr = −53±3%), after occlusion of the portal branch. In group Ia, %ΔLDFmax was −68±22%. This was significantly greater than in group Ib (42±17%), and the recovery of LDF during occlusion was greater in group Ia than in group Ib. %ΔLDFavr was −36±27% in the group with tumors, and this decrease was significantly smaller than in control group.

The LDF of the lobe mass increased after occlusion of the portal branch. The rate of increase was greatest in the control group (%ΔLDFavr = 284±124%), and smallest in group II (%ΔLDFavr = 77±60%). There was also a significant difference between group Ia (%ΔLDFavr = 199±54%) and group Ib (%ΔLDFavr = 115±58%).

The LDF of the tumor decreased after occlusion of the portal branch. The maximum decrease (35±23%) occurred at 40 sec after occlusion and then the LDF recovered slightly (%ΔLDFavr = −23±24%). The rate of decrease was greater in group Ia (%ΔLDFavr = −36±10%) than in group Ib (%ΔLDFavr = −11±17%), and the difference between them was significant.

5. Mean arterial pressure

Mean arterial blood pressure just before occlusion of a vessel was 97±10 mmHg in the control group and 94±9 mmHg in animals with tumors; 97±9 mmHg in group Ia and 93±6 mmHg in group Ib; and 94±7 mmHg in group I and 92±12 mmHg in group II. Mean arterial blood pressure did not change after occlusion of the hepatic arterial branch in most experiments, but in a

few experiments there was a slight, transient rise just after occlusion, probably due to a neural reflex. Mean arterial blood pressure did not change after occlusion of the portal branch in most experiments.

B. Experiment 2

1. Tumor size

The diameter of tumors measured after hemodynamic study was 10–23 mm (16.6±4.1 mm).

2. LDF of normal liver tissue in dead rabbits

The LDF of 7 post-mortem livers was 20±7 mV, and we assumed that 20 mV was the true zero value for blood flow in experiment 2.

To determine the rates of LDF changes in response to vasoactive agents, we used the maximum among average rates for every two minutes during infusion.

3. Basal LDF of normal liver tissue and tumor

The basal LDF just before infusion of vasoactive substances was 117±36 mV in normal liver tissue of the main lobe and 130±70 mV in tumors.

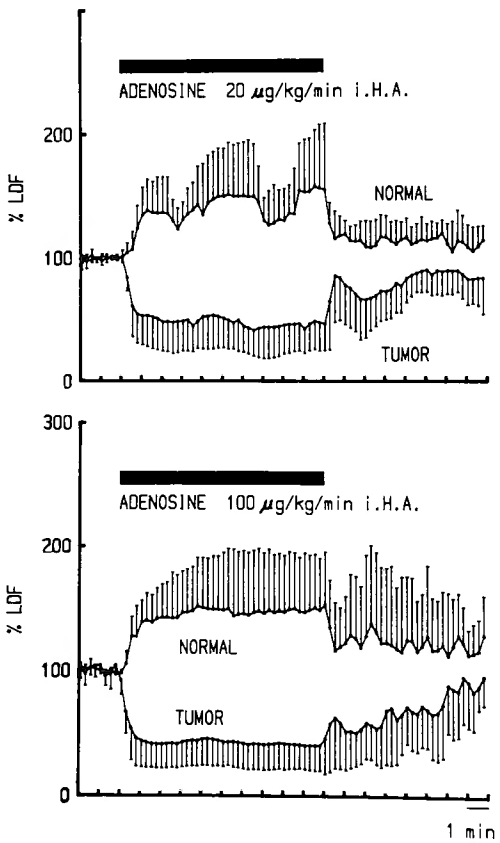

Fig. 6 Mean %LDF curves of normal liver tissue and a tumor, with intrahepatic arterial infusion of 20 μg/kg/min and 100 μg/kg/min of adenosine.

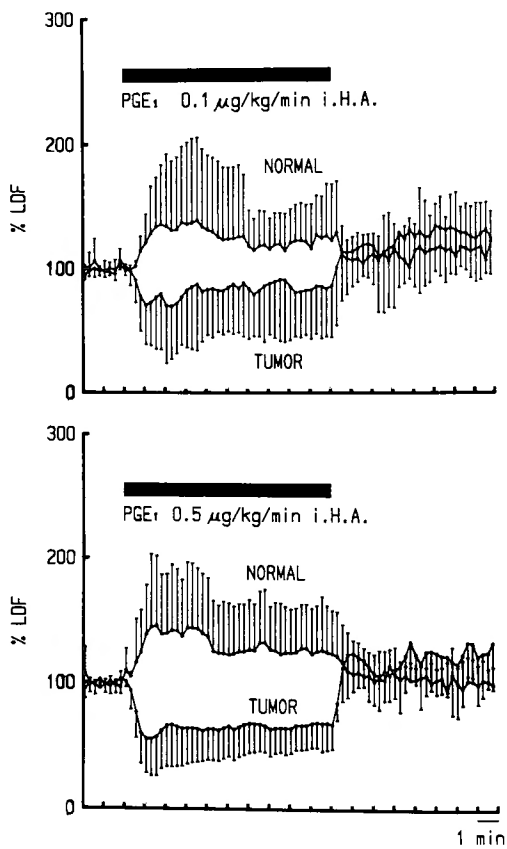

Fig. 7 Mean %LDF curves of normal liver tissue and a tumor, with intrahepatic arterial infusion of 0.1 μg/kg/min and 0.5 μg/kg/min of prostaglandin E_1.

4. Effect of adenosine (Fig. 6)

When adenosine was infused into the hepatic artery, the LDF of normal liver tissue increased and the LDF of tumors decreased, and this changes persisted without tachyphylaxis until the infusion was stopped, as shown in Fig. 6. Rates of increase of normal liver tissue LDF with 20 μg/kg/min and 100 μg/kg/min of adenosine were 48% and 49%, respectively, and rates of decrease of tumor LDF were 56% and 59%, respectively.

5. Effect of prostaglandin E_1 (Fig. 7)

When prostaglandin E_1 was infused into the hepatic artery, the LDF of normal liver tissue increased and the LDF of tumors decreased, as with adenosine, but there were more fluctuations and some recovery as shown in Fig. 7. Rates of increase of normal liver tissue LDF with 0.1 μg/kg/min and 0.5 μg/kg/min of prostaglandin E_1 were 21% and 36% respectively, and rates of decrease of tumor LDF were 35% and 41%, respectively.

6. Effect of angiotensin II (Fig. 8, 9)

When angiotensin II was infused into the hepatic artery, the LDF of normal liver tissue decreased, but then recovered especially with 1.0 μg/kg/min. Rates of decrease were 14% and 18% with

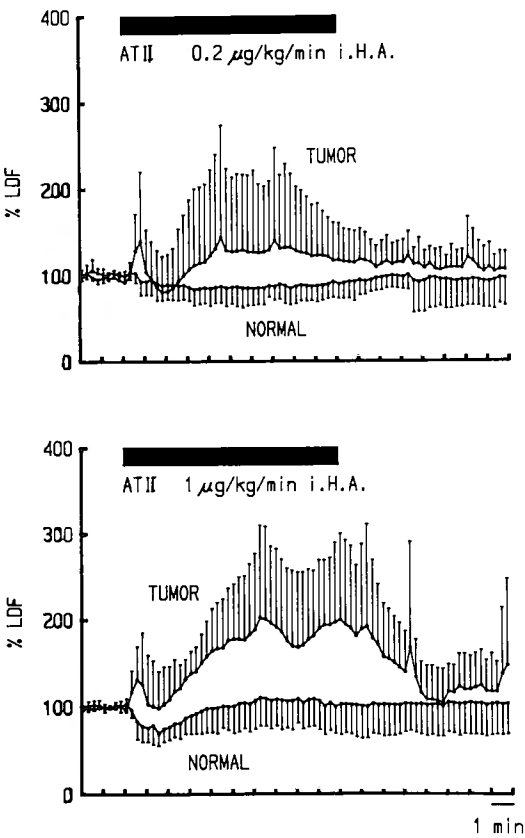

Fig. 8 Mean %LDF curves of normal liver tissue and a tumor, with intrahepatic arterial infusion of 0.2 μg/kg/min and 1.0 μg/kg/min of angiotensin II.

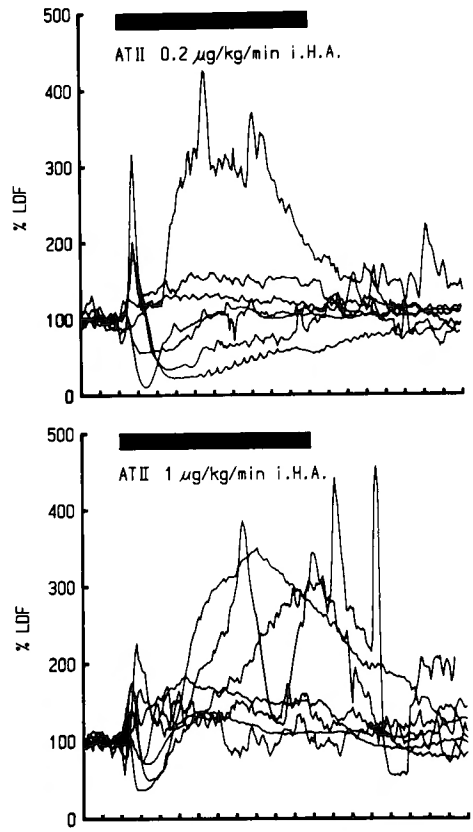

Fig. 9 %LDF curves of a tumor, with intrahepatic arterial infusion of 0.2 μg/kg/min and 1.0 μg/kg/min of angiotensin II.

0.2 μg/kg/min and 1.0 μg/kg/min of angiotensin II, respectively.

Tumor LDF increased with angiotensin II in most rabbits, but there was considerable fluctuation, rates of increase varied widely, and in some rabbits LDF decreased after an initial rise during infusion especially with 0.2 μg/kg/min of angiotensin II (Fig. 9). Rates of tumor LDF increase were 31% and 87% with 0.2 μg/kg/min and 1.0 μg/kg/min of angiotensin II, respectively.

7. Mean arterial pressure

Mean arterial blood pressure in the basal state was 85±8 mmHg. It did not change with adenosine or prostaglandin E_1, but increased with angiotensin II. This increase persisted during infusion of 0.2 μg/kg/min and 1.0 μg/kg/min of angiotensin II, by 6% and 16%, respectively.

Discussion

Ligation of the hepatic arterial branch or portal branch has been used to treat various liver tumors, including hemangioma, hepatoma and metastatic tumors (8, 11). Recently, transhepatic arterial embolization (21) has been increasingly performed, and percutaneous transhepatic portal embolization (15) has also been used. Changes in hepatic circulation following these procedures have been studied by morphological, physiological and pharmacological methods (3, 12). This time, we used laser-Doppler flowmetry to study the acute effects of these procedures, and to elucidate the relationships between hepatic arterial and portal blood flow in the microcirculation of normal liver tissue and tumors.

This technique allows noninvasive, continuous measurement of microcirculation over 1 mm^3 of surface area in a variety of tissues, and is also very sensitive to small changes in blood flow (5, 10). Though *Shepherd* et al, reported that laser-Doppler flowmetry gave unreproducible results in a perfusion study of isolated rat livers (18), this may have been due to the heterogeneity of the liver microcirculation (4). In the present study, the basal LDF of normal liver tissue ranged from 34 mV to 259 mV The LDF at one site in normal liver tissue was quite different from the LDF at another site several millimeters away. Tumor LDF was much more variable than normal liver LDF, and ranged from 45 mV to 661 mV This heterogeneity has been thought to be a characteristic of tumor vasculature.

Since the study by *Burton-Opitz*, many investigators have reported on the relationship between hepatic arterial blood flow and portal blood flow, and there is some agreement that the hepatic arterial blood flow changes to compensate for changes in the portal blood flow, while the portal blood flow is not altered by changes in hepatic arterial blood flow (though intrahepatic portal resistance might be altered) (9). *Lautt* called this the "hepatic arterial buffer response" (13). Many possible mechanisms of this response have been presented. According to *Lautt*, these include a myogenic theory, a physical mechanical interplay, a neural reflex, action of a vasoconstrictor in portal blood, a metabolic by-product entering sinusoidal blood, and the dilator (adenosine) washout theory presented by Lautt. Evaluating the present data based on this relationship, the decrease rate in the LDF of normal liver tissue in the main lobe after occlusion of the hepatic arterial branch represents the ratio of hepatic arterial blood flow in the liver microcirculation, and the decrease rate in the LDF of normal liver tissue after occlusion of the portal branch represents the subtraction of the hepatic arterial buffer response from the ratio of portal blood flow in the liver microcirculation. The partial recovery of normal liver tissue LDF in the main lobe seen after occlusion of the portal branch is probably due to this mechanism, and the time needed for the LDF to reach a plateau was about 45–100

sec.

This partial recovery of LDF was seen most clearly in the control group. It was less evident in group Ia and even more difficult to discern in group Ib. Because the decrease in the normal liver tissue LDF in the main lobe after occlusion of the hepatic arterial branch was smallest in the control group, larger in group Ia and largest in group Ib, the proportion of portal blood flow in the liver microcirculation was probably largest in the control group, smaller in group Ia and smallest in group Ib. This is supported by the fact that the increase in LDF of the lobe mass after occlusion of the portal branch was largest in the control group, smaller in group Ia and smallest in group Ib. The fact that the strength of the hepatic arterial buffer response is said to be proportional to the reduction in portal blood flow, is consistent with the fact that the partial recovery of LDF after occlusion of the portal branch was greatest in the control group and smallest in group Ib.

As stated above, the proportion of portal blood flow in the normal liver microcirculation was greatest in the control group, smaller in group Ia and smallest in group Ib, that is, the proportion of portal blood flow decreased with tumor growth. The reason for this is unclear, but the portal branch may have been compressed by the tumor and it may have contained tumor thrombi.

It has been generally accepted that liver tumors are supplied with blood flow predominantly from the hepatic artery, but there is also some blood from the portal vein at the margins of the tumor or when a tumor is very small (2). From the present data we cannot determine whether the tumor received some portal blood or not, because the zero level of the LD5000 was not exact.

The tumor LDF decreased after occlusion of the portal branch. Ackerman also reported this phenomenon (3). This can be explained as follows. With occlusion of the portal branch, hepatic arteriolar resistance in the normal tissue decreases because of the hepatic arterial buffer response, and this brings about the redistribution of the hepatic arterial blood from tumor tissue to normal liver tissue, so microcirculation in the tumor decreased. The fact that the decrease was greater in group Ia than group Ib supports this explanation, because the proportion of portal blood flow in normal liver tissue was higher in group Ia than in group Ib, and the volume of normal liver tissue compared to tumor volume was greater in group Ia than in group Ib. According to this hypothesis, portal ligation for the treatment of a liver tumor would be more effective, at least in the acute phase, for a small tumor and in a liver in which the portal circulation is well maintained.

There was no difference in the hemodynamic changes between group I and group II, except for the LDF changes of the lobe mass. In group II, in which Vx2 tumors were implanted through the portal vein as a model of multiple metastatic tumors, the increased LDF of the lobe mass after occlusion of the portal branch to the main lobe was smaller than in group I. This was probably due to increased intrahepatic portal resistance caused by the presence of a tumor in the lobe mass in group II.

It has been pointed out that tumor vessels are morphologically and functionally quite different from normal vessels. These specific characteristics have been used to treat malignant tumors. We studied the effect of intrahepatic arterial infusion of adenosine, prostaglandin E_1 and angiotensin II on microcirculation in normal liver tissue and in tumors.

Adenosine, which *Lautt* assumed to be the intrinsic mediator of the hepatic arterial buffer response (14), is a potent dilator of arterioles. In our study, 100 μg/kg/min of adenosine increased the LDF of normal liver tissue by 49%. If we suppose that the ratio of hepatic arterial blood flow in tissue microcirculation is 37% (from the result of the occlusion of the hepatic arterial branch in group Ia), and that the portal blood flow did not change during infusion of adenosine, the increase in hepatic arterial blood flow was 136%. In contrast, tumor LDF decreased with adenosine infusion as

a mirror image of the LDF in normal liver tissue. This was probably due to redistribution of flow from tumor tissue to normal liver tissue caused by the decrease in hepatic arteriolar resistance of normal liver tissue.

Prostaglandin E_1 has an effect similar to that of adenosine, but 100 μg/kg/min of adenosine was more potent and resulted in a persistent change in blood flow than did 0.5 μg/kg/min of prostaglandin E_1.

On the other hand, Angiotensin II, a potent constrictor of arterioles, decreased the LDF of normal liver tissue. With 1.0 μg/kg/min of angiotensin II, the maximum decrease was seen 2–3 min after the infusion began, and there was recovery during infusion, almost to the basal LDF. This can be explained as a systemic effect of angiotensin II, since systemic arterial pressure rose by 16% with 1.0 μg/kg/min of angiotensin II. Another possible explanation is the escape phenomenon or tachyphylaxis to angiotensin II, though *Richardson and Withrington* reported that in the dog there was no tachyphylaxis to angiotensin II in the hepatic arterial vascular bed while there was marked tachyphylaxis in the portal vascular bed (17). Tumor blood flow was increased, both by the redistribution of flow from normal tissue to tumor due to hepatic arteriolar vasoconstriction in normal tissue, and also by passive dilation of tumor vessels with increased blood pressure, because tumor vessels lack autoregulation.

Angiotensin II has been used with anticancer agents to selectively increase the uptake of these agents by tumor tissue, i.e. "hypertensive chemotherapy" (20). On the other hand, adenosine can be used in combination with hyperthermia. Selective heating of a tumor could be reinforced with adenosine, because it would slow down the blood flow to the tumor and increase the blood flow to normal tissue. Thermal damage to normal tissue will be decreased by increasing the blood flow to normal tissue, unlike with the combination of hyperthermia and TAE (7).

There are many ways to treat liver tumors, and combined or multimodality therapy is often required. When considering treatment, it is important to understand the microcirculation and vasculature of the liver and of intrahepatic tumors. Laser-Doppler flowmetry will be useful for evaluating the hemodynamics of liver and liver tumors.

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和文抄録

レーザー・ドップラー血流計による Vx2 癌移植家兎肝における微小循環動態の研究

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Vx2 癌移植家兎肝の肝組織正常部および腫瘍部の微小循環血流をレーザー・ドップラー血流計で測定し，肝動脈枝遮断，門脈枝遮断による変動（実験1）と血管作動性物質の肝動脈内注入による変動(実験2)を検討した．

【実験1】 肝 main lobe に注ぐ肝動脈枝の遮断にて main lobe 正常部血流はコントロール群で 11±11%，Ia 群（腫瘍径 10–20 mm）で 37±37%，Ib 群（腫瘍径 25–50 mm）で 49±37%低下し，腫瘍の増大とともに，腫瘍周囲の正常部組織血流における門脈成分の減少を認めた．腫瘍部血流は肝動脈枝遮断により 88±13%低下した．main lobe への門脈枝の遮断にて，main lobe 正常部血流は最初急激に低下した後少し回復する傾向をコントロール群＞Ia 群＞Ib 群の順に認め，門脈血流の減少に対する肝動脈血流の代償性増加 (hepatic arterial buffer response) によると思われた．腫瘍部血流は門脈枝遮断により Ia 群で 36±10%，Ib 群で 11±17%低下し，肝動脈血流の代償作用を介した，肝動脈血流の腫瘍部から正常部への再分布によるものと思われた．腫瘍を直接肝に移植した群（I群）と経門脈的に移植した群（II群）とでは，肝動脈枝遮断，門脈枝遮断に対する変化に差は認めなかった．

【実験2】 adenosine 20 μg/kg/min, 100 μg/kg/min の肝動脈内注入にて肝正常部血流は48%，49%の増加を，腫瘍部血流は56%，59%の低下を示した．prostaglandin E_1 0.1 μg/kg/min, 0.5 μg/kg/min では正常部血流は21%，36%の増加を，腫瘍部血流は35%，41%の低下をみた．angiotensin II 0.2 μg/kg/min, 1.0 μg/kg/min では正常部血流は14%，18%の低下を，腫瘍部血流は31%，87%の増加を示したが，ばらつきが大きかった．adenosine は温熱療法の際の腫瘍部の選択的加温の増強に有用と思われた．